Gentianae Macrophyllae Radix

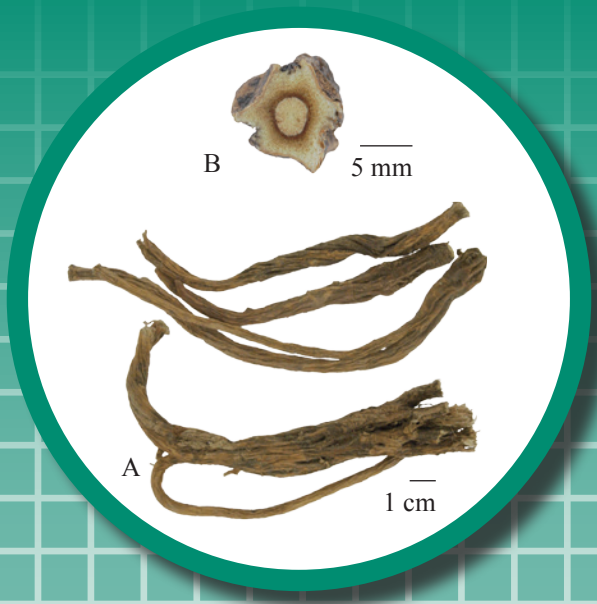


Figure 1 (i) A photograph of dried root of *Gentiana macrophylla* Pall.

A. Root B. Magnified transverse section of root

Figure 1 (ii) A photograph of dried root of *Gentiana straminea* Maxim.

A. Root B. Magnified transverse section of root

1. NAMES

Official Name: Gentianae Macrophyllae Radix

Chinese Name: 秦艽

Chinese Phonetic Name: Qinjiao

2. SOURCE

Gentianae Macrophyllae Radix is the dried root of *Gentiana macrophylla* Pall. or *Gentiana straminea* Maxim. (Gentianaceae). According to the description, the former species is known as "Qinjiao" and the latter is known as "Mahuajiao". The root is collected in spring and autumn, foreign matter removed, dried under the sun to obtain Gentianae Macrophyllae Radix.

3. DESCRIPTION

***Gentiana macrophylla* Pall.:** Subcylindrical, the upper part thick and the lower part thin, twisted, 10-30 cm long, 10-30 mm in diameter. Externally yellowish-brown to greyish-yellow, with longitudinal or twisted wrinkles, remnants of stem bases and fibrous leaf sheath occurring at the apex. Texture hard and fragile, easily broken. Fracture slightly oily, bark yellow or brownish-yellow, wood yellow. Odour characteristic; taste bitter and slightly astringent [Fig. 1 (i)].

***Gentiana straminea* Maxim.:** Subconical, usually expanded in size by several entangled small roots, up to 70 mm in diameter. Externally dark brown, rough, with fissures showing reticulate-pitted striations. Texture lax and fragile, easily broken. Fracture usually in the shape of rotten-wood [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

***Gentiana macrophylla* Pall.:** Outer periderm and inner periderm cork cells subrectangular, tangentially prolonged. Some cork cells divided into 2-10 daughter cells. Groups of sieve tubes scattered in phloem. Cambium in a ring. Xylem vessels abundant, 10-70 μm in diameter [Fig. 2 (i)].

***Gentiana straminea* Maxim.:** Xylem usually eccentrically distributed; vessels 8-70 μm in diameter [Fig. 2 (ii)].

Powder

Colour yellowish-brown. Cork cells polygonal, subsquare, rectangular or irregular, each cell divided into 2-10 small and irregular-shaped cells, separator line visible. Vessels spiral or reticulate, 8-70 μm in diameter. Crystals of calcium oxalate rod-shaped, tiny, 1-7 μm long [Fig. 3 (i) and (ii)].

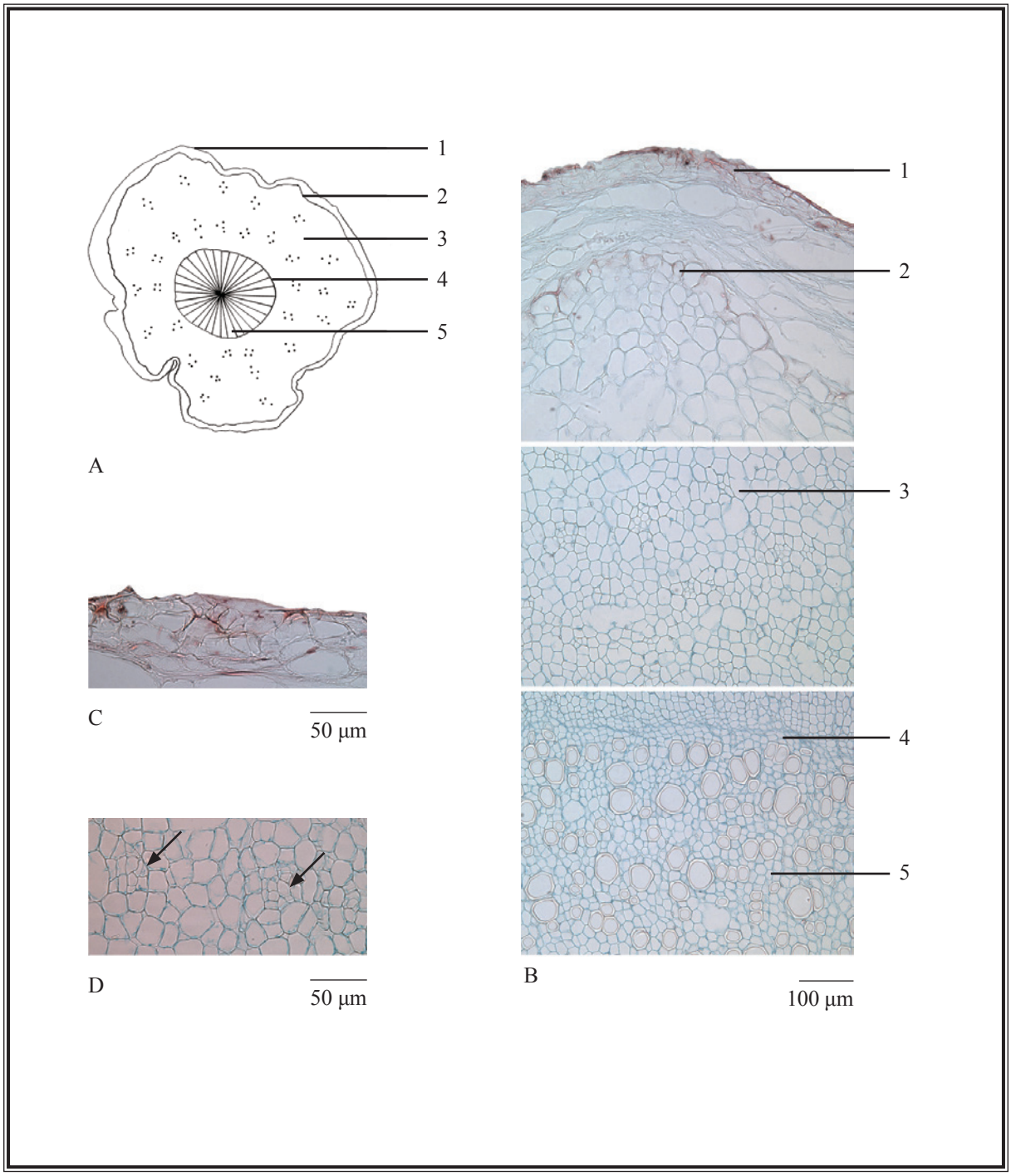


Figure 2 (i) Microscopic features of transverse section of dried root of *Gentiana macrophylla* Pall.

A. Sketch B. Section illustration C. Outer periderm cork cells D. Sieve tubes

1. Outer periderm 2. Inner periderm 3. Phloem 4. Cambium 5. Xylem

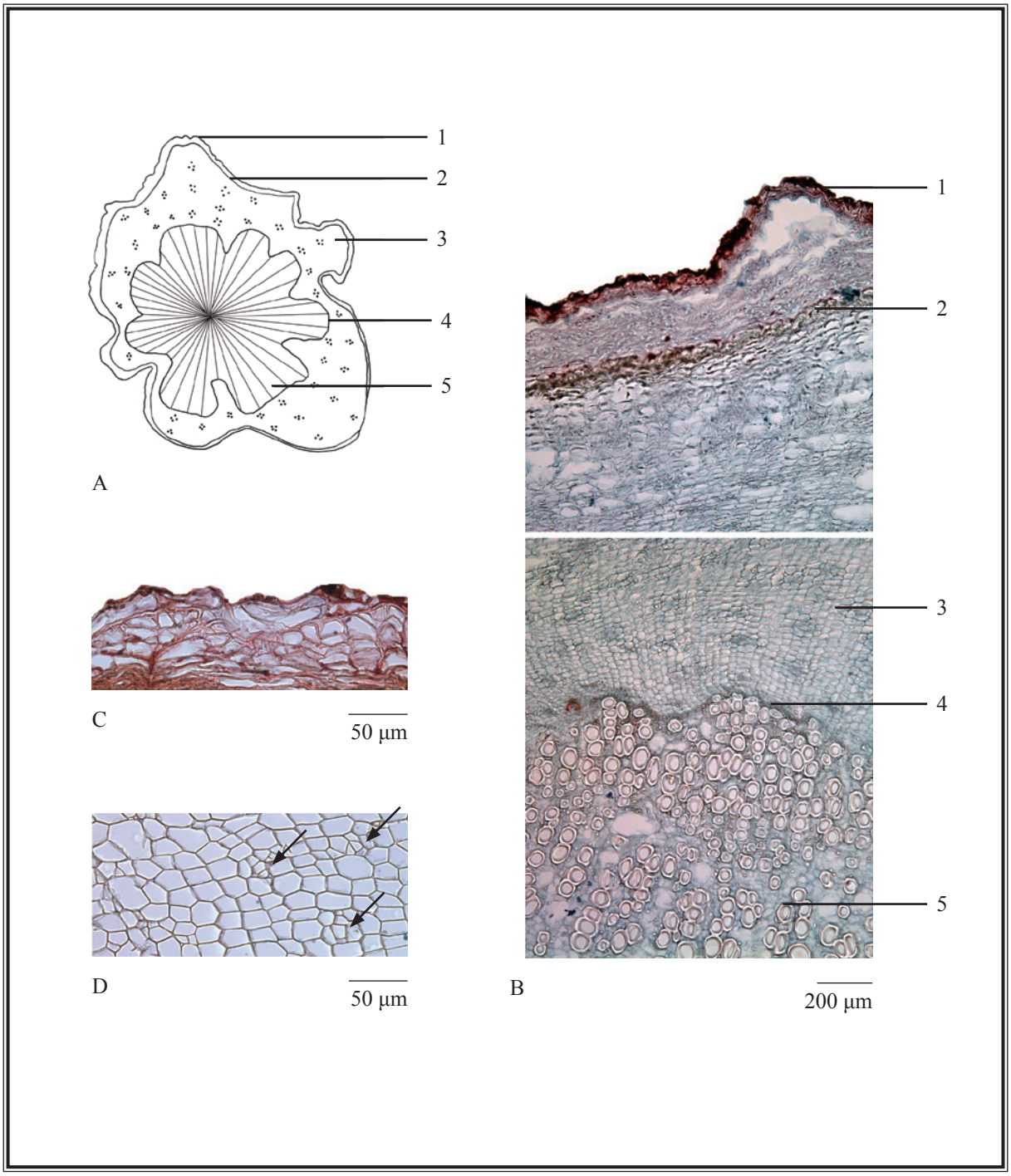


Figure 2 (ii) Microscopic features of transverse section of dried root of *Gentiana straminea* Maxim.

A. Sketch B. Section illustration C. Outer periderm cork cells D. Sieve tubes

1. Outer periderm 2. Inner periderm 3. Phloem 4. Cambium 5. Xylem

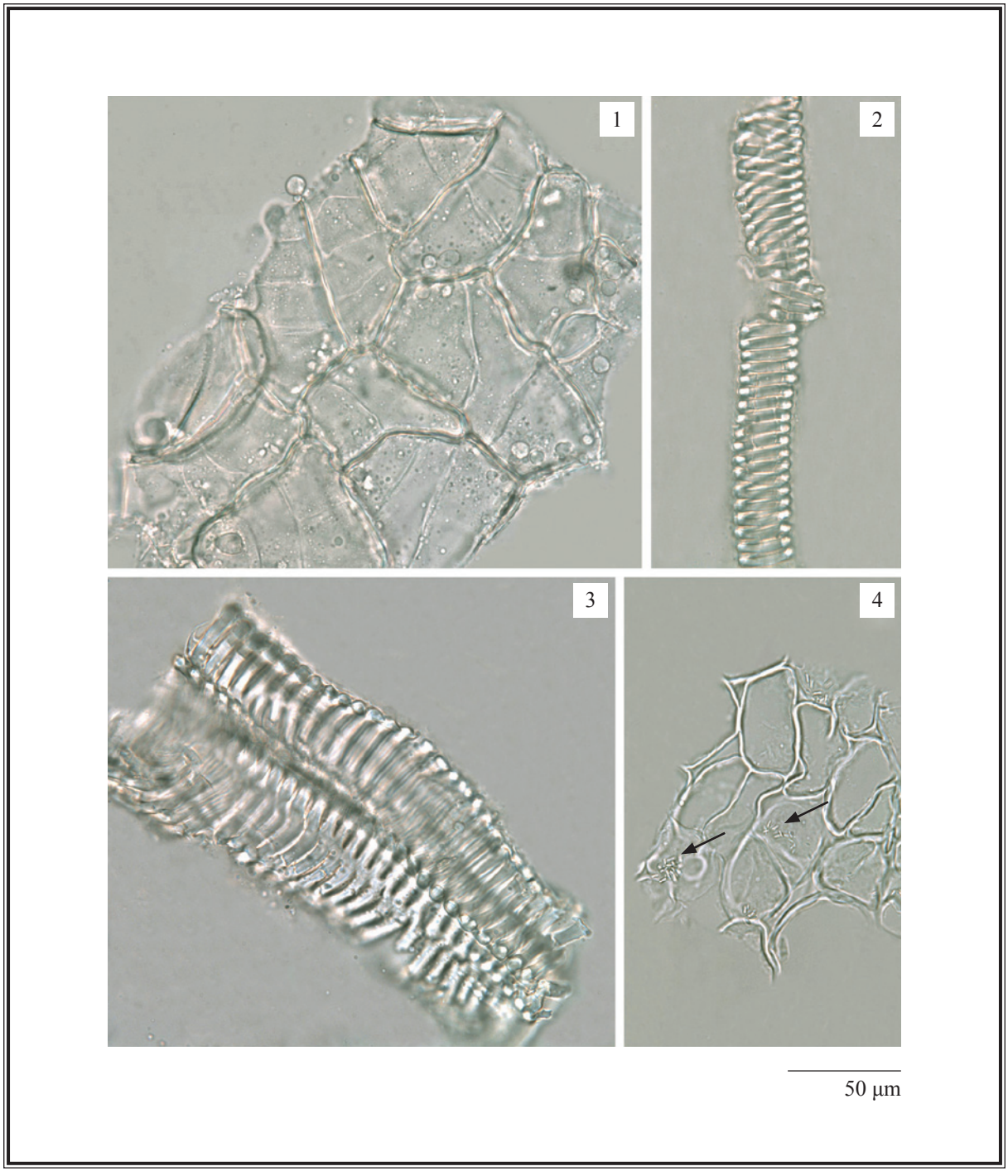


Figure 3 (i) Microscopic features of powder of dried root of *Gentiana macrophylla* Pall. (under the light microscope)

1. Cork cells 2. Spiral vessel 3. Reticulate vessels 4. Crystals of calcium oxalate

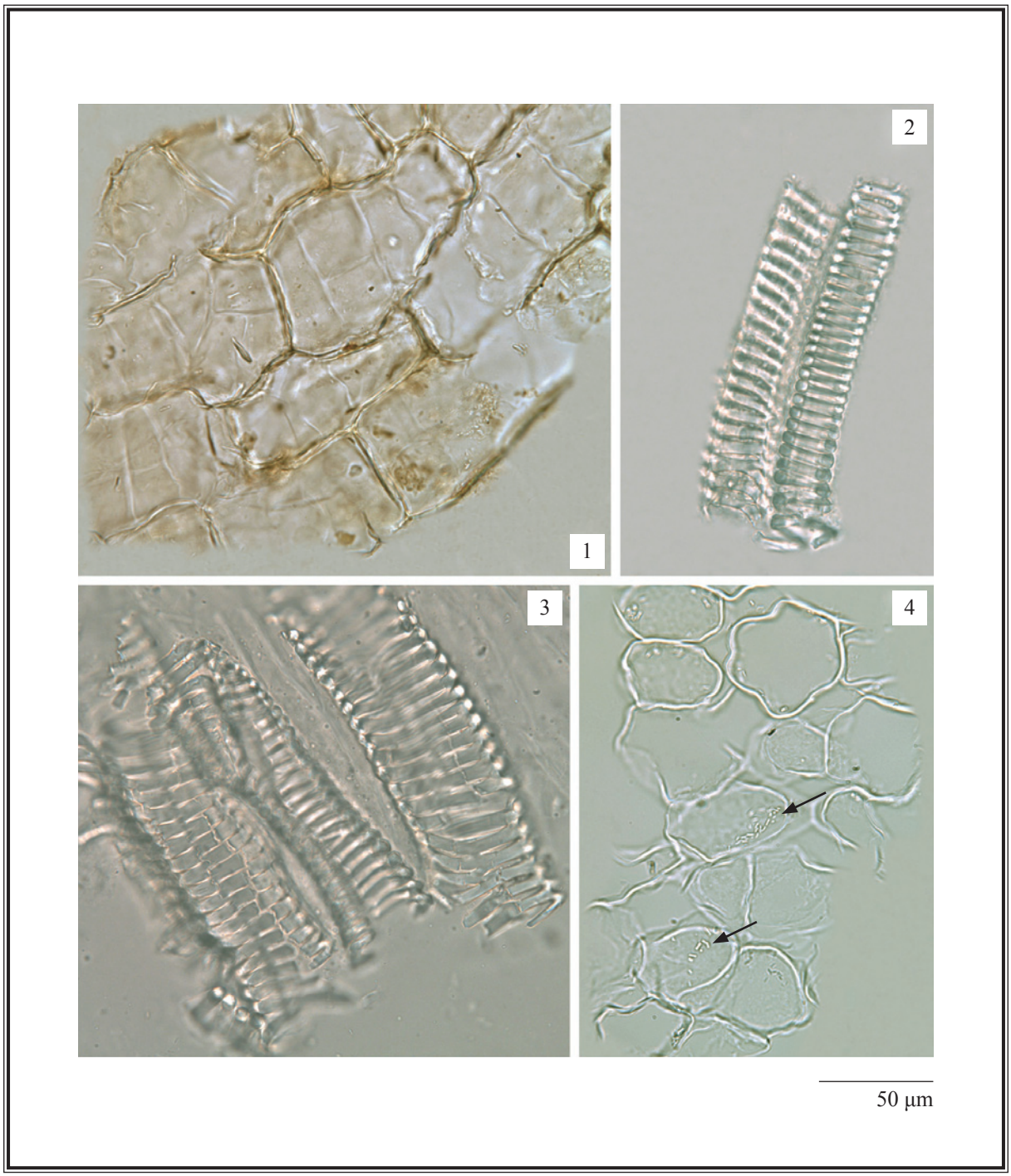


Figure 3 (ii) Microscopic features of powder of dried root of *Gentiana straminea* Maxim. (under the light microscope)

1. Cork cells 2. Spiral vessel 3. Reticulate vessels 4. Crystals of calcium oxalate

Footnote: Microscopic features of powder have no significant differences between the dried root of *Gentiana macrophylla* Pall. and *Gentiana straminea* Maxim.

4.2 Thin-Layer Chromatographic Identification *[Appendix IV(A)]*

Standard solutions

Gentiopicroside standard solution

Weigh 1.0 mg of gentiopicroside CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Loganic acid standard solution

Weigh 1.0 mg of loganic acid CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Developing solvent system

Prepare a mixture of ethyl acetate, ethanol, water and glacial acetic acid (5:1:0.5:0.1, v/v).

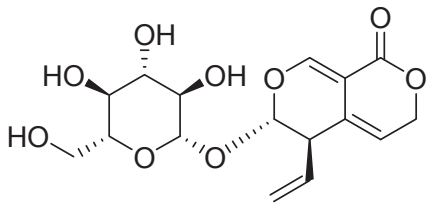
Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 5 mL of ethanol. Sonicate (270 W) the mixture for 15 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately gentiopicroside standard solution (1 μL), loganic acid standard solution (2 μL) and the test solution (1 μL) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate for development. Develop over a path of about 5 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)

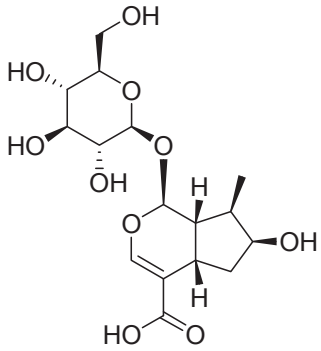


Figure 4 Chemical structures of (i) gentiopicroside and (ii) loganic acid

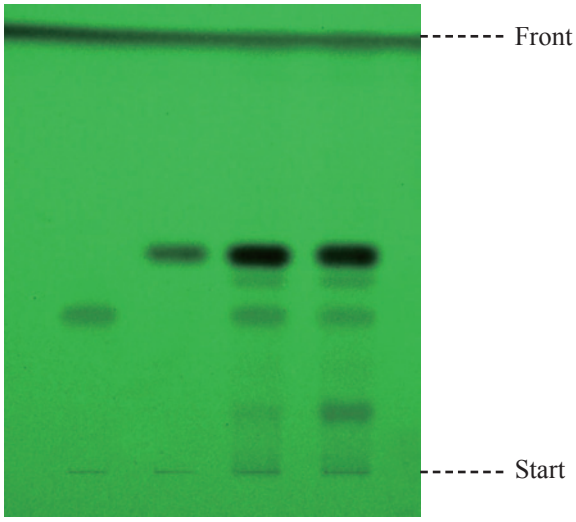


Figure 5 A reference HPTLC chromatogram of Gentianae Macrophyllae Radix extract observed under UV light (254 nm)

1. Loganic acid standard solution
2. Gentiopicroside standard solution
3. Test solution of
(i) dried root of *Gentiana macrophylla* Pall.
(ii) dried root of *Gentiana straminea* Maxim.

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of gentiopicroside and loganic acid (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting *(Appendix XII)*

Standard solutions

Gentiopicroside standard solution for fingerprinting, Std-FP (800 mg/L)

Weigh 8.0 mg of gentiopicroside CRS and dissolve in 10 mL of water.

Loganic acid standard solution for fingerprinting, Std-FP (300 mg/L)

Weigh 3.0 mg of loganic acid CRS and dissolve in 10 mL of water.

Test solution

Weigh 0.25 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of water. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with water. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.1% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 60	95 → 85	5 → 15	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of gentiopicroside Std-FP and loganic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of gentiopicroside and loganic acid should not be more than 5.0%; the RSD of the retention times of gentiopicroside and loganic acid peaks should not be more than 2.0%; the column efficiencies determined from gentiopicroside and loganic acid peaks should not be less than 35000 and 20000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and the *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 [Fig. 6 (i) or (ii)].

Procedure

Separately inject gentiopicroside Std-FP, loganic acid Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention times of gentiopicroside and loganic acid peaks in the chromatograms of gentiopicroside Std-FP, loganic acid Std-FP and the retention times of the four characteristic peaks [Fig. 6 (i) or (ii)] in the chromatogram of the test solution. Identify gentiopicroside and loganic acid peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of gentiopicroside Std-FP and loganic acid Std-FP. The retention times of gentiopicroside and loganic acid peaks in the chromatograms of the test solution and the corresponding Std-FP should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the four characteristic peaks of Gentianae Macrophyllae Radix extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Gentianae Macrophyllae Radix extract

Peak No.	RRT	Acceptable Range
1 (marker 1, loganic acid)	1.00	-
2	1.29 (vs peak 1)	± 0.03
3	1.34 (vs peak 1)	± 0.03
4 (marker 2, gentiopicroside)	1.00	-

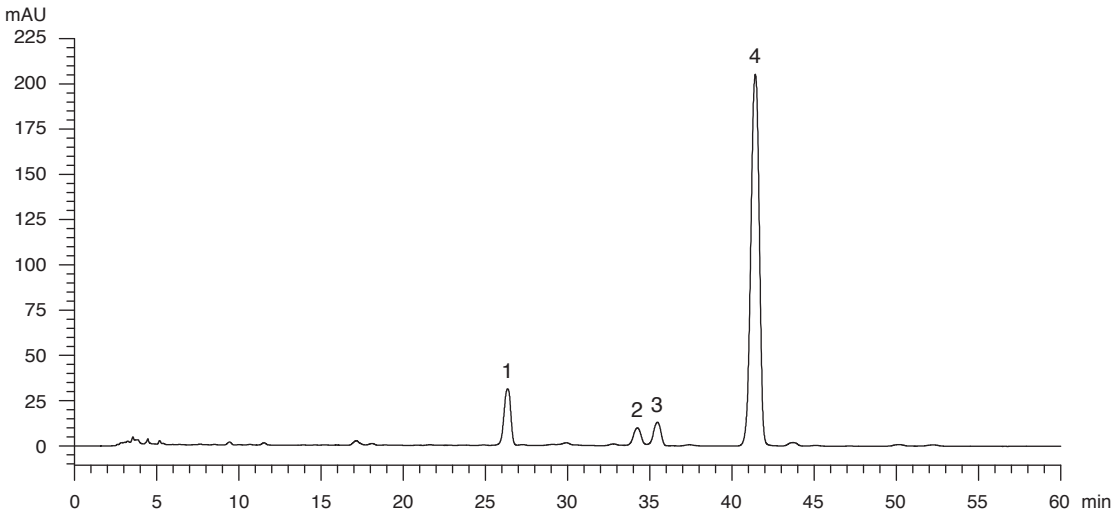


Figure 6 (i) A reference fingerprint chromatogram of dried root of *Gentiana macrophylla* Pall. extract

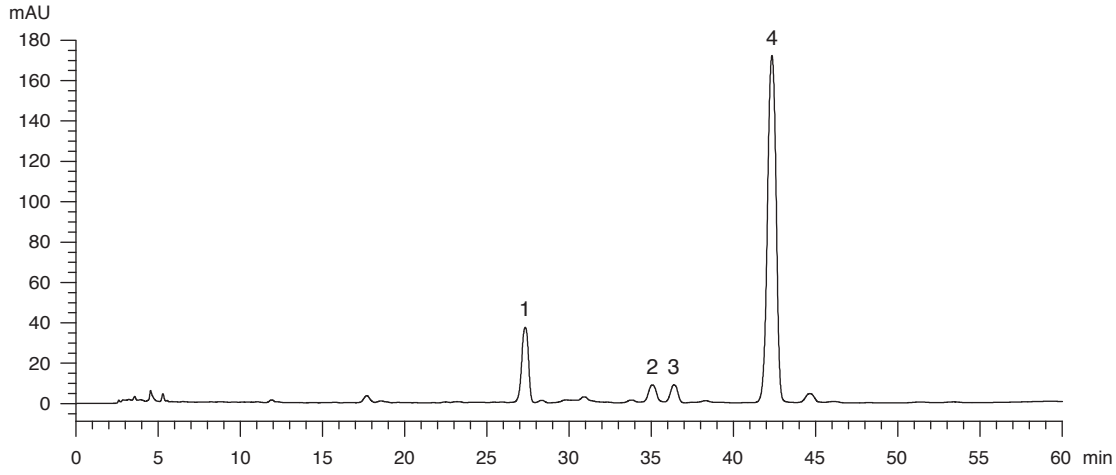


Figure 6 (ii) A reference fingerprint chromatogram of dried root of *Gentiana straminea* Maxim. extract

For positive identification, the sample must give the above four characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the respective reference fingerprint chromatograms [Fig. 6 (i) or (ii)].

5. TESTS

5.1 Heavy Metals *(Appendix V)*: meet the requirements.

5.2 Pesticide Residues *(Appendix VI)*: meet the requirements.

5.3 Mycotoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.5%.
Acid-insoluble ash: not more than 3.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 27.0%.
Ethanol-soluble extractives (cold extraction method): not less than 26.0%.

7. ASSAY

Carry out the method as directed in Appendix IV (B).

Standard solution

Mixed gentiopicroside and loganic acid standard stock solution, Std-Stock (1800 mg/L for gentiopicroside and 675 mg/L for loganic acid)

Weigh accurately 18.0 mg of gentiopicroside CRS and 6.75 mg of loganic acid CRS, and dissolve in 10 mL of water.

Mixed gentiopicroside and loganic acid standard solution for assay, Std-AS

Measure accurately the volume of the mixed gentiopicroside and loganic acid Std-Stock, dilute with water to produce a series of solutions of 20, 200, 400, 800, 1200 mg/L for gentiopicroside and 7.5, 75, 150, 300, 450 mg/L for loganic acid.

Test solution

Weigh accurately 0.25 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of water. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with water. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 60	95 → 85	5 → 15	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of the mixed gentiopicroside and loganic acid Std-AS (400 mg/L for gentiopicroside and 150 mg/L for loganic acid). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of gentiopicroside and loganic acid should not be more than 5.0%; the RSD of the retention times of gentiopicroside and loganic acid peaks should not be more than 2.0%; the column efficiencies determined from gentiopicroside and loganic acid peaks should not be less than 35000 and 20000 theoretical plates respectively.

The *R* value between gentiopicroside peak and the closest peak; and the *R* value between loganic acid peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed gentiopicroside and loganic acid Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of gentiopicroside and loganic acid against the corresponding concentrations of the mixed gentiopicroside and loganic acid Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify gentiopicroside and loganic acid peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed gentiopicroside and loganic acid Std-AS. The retention times of gentiopicroside and loganic acid peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of gentiopicroside and loganic acid in the test solution, and calculate the percentage contents of gentiopicroside and loganic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 3.7% of gentiopicroside ($C_{16}H_{20}O_9$) and not less than1.5% of loganic acid ($C_{16}H_{24}O_{10}$), calculated with reference to the dried substance.